Effect of Estrogen and Food Hardness on Metabolism and Turnover of Condylar Cartilage

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Aims: To clarify the effect of estrogen and food hardness on condylar cartilage and the cartilage-bone interface. **Methods:** A total of 56 rats were divided into four groups: *(1)* ovariectomized rats fed a normal (pressed pellet) food, *(2)* ovariectomized rats fed a soft (powder) food, *(3)* control rats fed a normal (pressed pellet) food, and *(4)* control rats fed a soft (powder) food. Some rats (n = 29) were sacrificed at the age of 67 days and others (n = 27) at the age of 87 days, and then 5-µm-thick sagittal paraffin sections were prepared from each temporomandibular joint (TMJ). Toluidine blue staining, in situ hybridization with type X collagen, terminal deoxynucleotidyl transferase and deoxyuridine triphosphate nick end labeling (TUNEL-assay), and tartrate-resistant acid phosphatase (TRAP) histochemistry were performed. Immunohistochemical analyses included cathepsin K, adiponectin, proliferating cell nuclear antigen (PCNA), and type X collagen staining. Analysis of variance and appropriate post-hoc tests were used in all analyses. **Results:** Ovariectomy and normal food consistency increased the thickness of condylar cartilage ($P < .001$), PCNA expression ($P < .001$) and type X collagen expression ($P < .001$). Ovariectomy decreased the number ($P < .05$) and size of osteoclasts ($P < .01$). Soft food increased the number of cartilage cells stained positively against adiponectin ($P < .01$). **Conclusion:** Decreased estrogen level and normal food hardness increase the thickness of condylar cartilage by various mechanisms. *J Oral Facial Pain Headache 2015;29:297–307. doi: 10.11607/ofph.1287*

Keywords: *adiponectin, estrogen, food hardness, loading, mandibular condylar cartilage, osteoclast, PCNA, proliferation, TRAP, type X collagen*

Temporomandibular disorders (TMD) comprise a variety of clinical conditions involving pain and dysfunction in the masticatory system, including the soft and hard tissues in the temporomandibular joints (TMJs).[1,2] Epidemiologic studies have shown that females comprise the majority of TMD patients, having more TMD symptoms and clinical signs than men, and the symptoms occur usually at 35 to 45 years of age.[3–6]

It has been suggested that estrogen may have an important role in TMJ pathology. Estrogen is a major hormonal regulator of bone metabolism in both genders, and it affects numerous factors and routes involved in bone homeostasis regulation.[7] Several studies have suggested an active role of estrogen in condylar cartilage metabolism.[8–11] Estrogen supplementation can result in a significant decrease in condylar cartilage thickness,[9] and a lack of systemic estrogen (ovariectomy) may induce an increase in condylar cartilage thickness.[10,11] Loading seems to be important in preserving the ideal thickness and beneficial for extracellular matrix composition of condylar chondrocytes.[11] It has been shown that soft food reduces the proliferative activity of condylar cartilage.[12]

Estrogen is known to affect condylar cartilage through its specific hormone receptors—estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ).[9,10,13] Estrogen affects the chondrocytes by inhibiting chondrocyte proliferation and increasing the maturation of chondrocytes.[9] It is not clear whether estrogen affects condylar cartilage

Table 1 Distribution of Rats into Study Groups (OVX, Control) by Age and Food Type

Study group	Soft food (n)	Normal food (n)
OVX		
Age 67 days	9	6
Age 87 days	6	6
Control		
Age 67 days	6	8
Age 87 days	7	8

OVX = ovariectomized.

thickness by regulating osteoclasts—their formation, number, and/or activity—in the cartilage-bone interface. Osteoclasts secrete cathepsin K, which is one of the most important proteases involved in bone matrix degradation, and it can be used as a marker for active osteoclasts.[14,15] Type X collagen is synthesized by hypertrophic chondrocytes, and it is assumed to be a marker for mature condylar cartilage.[16] Type X collagen is suggested to act as a replaceable material in the growth of bone matrix, and therefore its expression is linked with endochondral ossification.[17]

The adipokine adiponectin is secreted into plasma by adipocytes of white adipose tissue, and there is evidence that adiponectin affects chondrocytes in cartilage and induces the secretion of many interleukins.[18] For this reason, adiponectin is believed to have some role in degenerative joint diseases and cartilage metabolism.[18] It has been noted that estrogen inhibits the production of adiponectin, and a decreased level of estrogen causes dysfunction of adipocytes.[19,20]

It has also been suggested that altered food hardness affects the level of masticatory muscle contraction, which creates distinct occlusal forces and alters the loading of the TMJ.[21–23] Although the effect of altered food hardness and estrogen on condylar cartilage has been under investigation in many studies,[10,24] it is not clear which factors transmit the effect of estrogen and loading. The aim of the present study was to clarify the effect of estrogen and food hardness on condylar cartilage and the cartilage-bone interface. Proliferative activity of cartilage cells, the number and size of osteoclasts, expression of type X collagen, the proportional amount of cartilage cells stained against adiponectin, and apoptosis of cartilage cells were investigated.

Materials and Methods

Animals and Experimental Groups

A total of 56 rats were divided into four groups. An ovariectomy was performed on rats in two experimental groups at the age of 60 days. The rats in the first experimental group were ovariectomized and fed a normal (pressed pellet) food (Landsmännen R36, Lactamin AB) throughout the experiment. The rats in the second experimental group were ovariectomized and fed a soft (powder) food throughout the experiment. The rats in the first control group were fed the same normal food as experimental rats were fed, and the rats in the second control group were fed the same soft food as the experimental rats throughout the animal experiment (Table 1).

Twenty-nine rats were sacrificed at the age of 67 days and 27 rats at the age of 87 days (Table 1). The animal experiments were approved by the National Animal Experiment Committee of Finland and determined to be ethically acceptable.

Tissue Preparation

The rats were sacrificed with carbon dioxide and then decapitated. The crania were fixed in 4% formalin for 24 hours. After fixation, the soft tissues, except for the articular capsule and surrounding muscles, were removed and the samples were decalcified in ethylenediaminetetraacetic acid (EDTA) and heated in a microwave for 100 hours at 37°C. After decalcification, the cranium was cut sagittally in half to separate the right and left TMJ. The sections were embedded in paraffin and cut into 5-µm sections in the sagittal direction.

Immunohistochemistry

The most central sagittal sections of the TMJ were chosen for all further analyses. Endogenous peroxidase activity was quenched by treating deparaffinized sections with 1% H_2O_2 for 30 minutes at room temperature. The samples for type X collagen and cathepsin K staining were pretreated with 0.4% pepsin for 60 minutes at 37°C. Nonspecific binding of antibodies was blocked by normal horse serum (diluted in 1:20 phosphate-buffed saline [PBS]).

The sections were preincubated with monoclonal type X collagen antibody (Quartett Immunodiagnostika & Biotechnologie GmbH, diluted 1:100 in PBS, 0.1% bovine serum albumin [BSA]) and cathepsin K (Biovendor, Brno, diluted 1:7,000 in PBS, 0.1% BSA) at +37°C for 30 minutes. Non-immune serum (DAKO), diluted in the same manner as the primary antibody, was applied for specimens chosen randomly to be negative controls. After preincubation, the sections were incubated overnight at +4°C. A secondary antibody, anti-mouse immunoglobulin G (IgG) produced in horse (Vector) was applied for 1 hour at room temperature, followed by Vectastain avidin-biotin-peroxidase complex (Vector) for 30 minutes at room temperature. The immunostaining was visualized with a DAB (3,3'-diaminobenzidine) peroxidase substrate kit (Vector). The sections were counterstained with Mayer's hematoxylin (Histolab Products AB).

Monoclonal mouse anti-proliferating cell nuclear antigen (PCNA) (AbD Serotec, diluted 1:800 in DAKO antibody diluent) and monoclonal mouse anti-adiponectin (Abcam, diluted 1:500 in DAKO antibody diluent) were used with a DAKO kit following the basic protocol. The sections for anti-adiponectin were treated at first with 0.4% pepsin at 37°C for 30 minutes. The sections for anti-PCNA did not require any pretreatment. Before applying the primary antibody to the samples, Background Sniper (Biocare Medical) was used on the specimens for 30 minutes at room temperature to reduce nonspecific staining. Primary antibody incubation for both antibodies was at room temperature for 30 minutes. For negative controls, DAKO non-immune serum was used.

In Situ Hybridization

Radioactive in situ hybridization on paraffin sections was carried out according to standard protocols.[25,26] 35S-UTP (uridine 5'-[a-thio] triphosphate-a-35S) labeled probes (Amersham) were used to detect the expression of type X collagen.[27] Exposure time was 14 days at +4°C.

Enzyme Histochemistry

A tartrate-resistant acid phosphatase (TRAP) kit (Sigma-Aldrich) was used to stain deparaffinized sections of the TMJ. The staining was performed by following the exact procedure within the kit.

TUNEL Labeling

A terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) apoptosis kit (Roche) was used to measure the amount of apoptotic cells in the TMJ samples. Deparaffinized sections of the condylar head were tested with three different pretreatments: 0.4% pepsin for 60 minutes at 37°C, with a citrate buffer for 25 minutes at 98°C in a microwave oven, and with an EDTA citrate solution in a microwave oven for 25 minutes. A negative control was prepared according to the instructions within the kit. A human colon cancer sample was used for a positive control.

For further analysis of apoptosis, monoclonal mouse anti-p53 clone DO-7 (DAKO) was applied to deparaffinized samples. Staining was performed according the standard DAKO kit procedure. Three different pretreatments, described above with TUNEL, were used. A human colon cancer sample was used for a positive control.

Histomorphometric Analysis

Deparaffinized sections of the condylar head were stained with toluidine blue. The most central sagittal section of the condylar head was chosen from all the stained samples and taken for histomorphometric analysis. The condylar head was divided sagittally into posterior, most superior, and anterior segments. The center point of the most superior segment was defined as the point where the articular disc was thinnest. The measurement points of the anterior and posterior segments of the condylar head were defined to be 1,000 µm from the center point of the most superior segment (Fig 1). The rationale for the 1000-µm distance was that thereby each condylar head was divided in the same way regardless of the shape of the condylar head. The thickness of condylar cartilage was measured at the center point of each of the three segments.

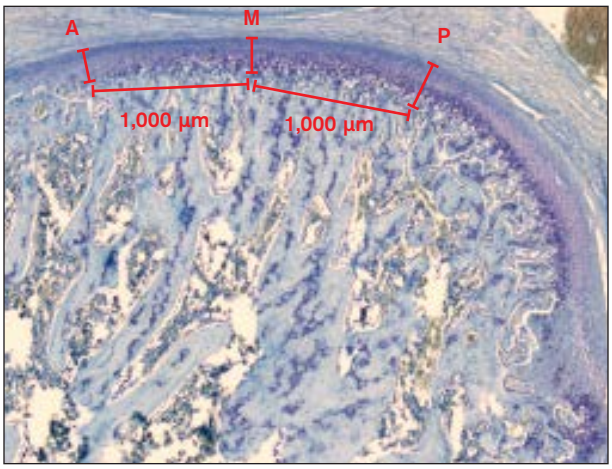


Fig 1 Most central sagittal section of a rat's condylar head stained with toluidine blue. Red lines indicate the central point of each of three segments of the condyle. (A) anterior segment; (M) most superior segment; (P) posterior segment. The thickness of the cartilage was measured at the central point of each segment.

The measurements were done with a microscope (Leica Leitz) and an image analyzer (ImageJ).

Microscopic Analysis

Analysis of type X collagen expression was performed with a microscope at 2.5× magnification (Leica Leitz) and an image analyzer (ImageJ). Condylar cartilage was first separated from the condylar head with ImageJ cutting tools. Immunostaining against type X collagen was then visualized by applying color filters and adjusting the threshold. The extracellular area (µm^2) stained against antibody was calculated automatically with ImageJ.

Immunohistochemical stainings against PCNA and adiponectin were analyzed in each of the three segments of the condylar head with a microscope at 20× magnification and an image analyzer. A defined frame of 500-µm width and unlimited height was applied on the center of each three segment of condylar head. The height of the frame was not defined because there was great variation in the thickness of condylar cartilage

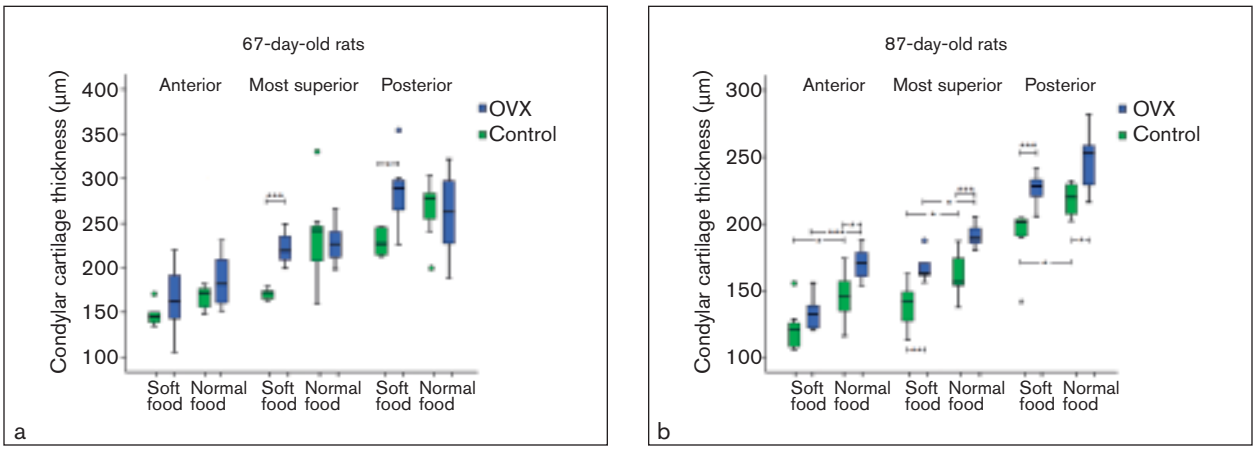


Fig 2 Box plot diagrams illustrating the thickness of condylar cartilage in **(a)** 67-day-old rats and **(b)** 87-day-old rats. *P < .05; **P < .01; ***P < .001. OVX = ovariectomized rats.

between specimens. Inside the frame, the number of positively and negatively stained cells in the condylar cartilage was registered. A proportional amount of staining was calculated by dividing the number of positively stained cells by the total number of cells.

The proportional expression of type X collagen mRNA was analyzed in situ in a similar method as PCNA and adiponectin. For the in situ hybridization analysis, the proportional expression and localization of type X collagen were evaluated.

Cathepsin K and TRAP analyses were performed with a microscope and an image analyzer (ImageJ). The image of the condylar head at the most central point (the point where the articular disc was thinnest) was taken with the same settings for each specimen to ensure that exactly the same portion of the condylar head was visible in all the images and they were therefore comparable. Magnification of 10× was used for this analysis; the image covered a large area of the condylar head and was accurate enough to perform the measurements. Each image was applied with a color filter and an adjusted threshold. The number of osteoclasts (cathepsin K and TRAP-positive cells) was measured automatically with ImageJ particle analysis. The analysis also registered the volume/area of each particle stained against antibody.

Statistical Analyses

Statistical analyses were done with SPSS (SPSS Inc). Analysis of variance analysis (ANOVA) was used in all analyses. Variables for the analysis were the thickness of condylar cartilage (µm), number of osteoclasts, average size of osteoclasts (µm^2), area stained against type X collagen (µm^2), proportional amount of cells stained against PCNA, and proportional amount of cells stained against adiponectin. Post-hoc tests were performed after ANOVA to verify which pairs of groups were significantly different. The normality of the data was examined with Shapiro-Wilk test. Tamhane's T2 was used as a post-hoc test with the data concerning the number and average size of osteoclasts (cathepsin K and TRAP) in 87-day-old rats, and Tukey HSD (Honestly Significant Difference) test was used with the 67-day-old rats. Tamhane test was chosen because the groups of 87-day-old rats did not have equal variances.[28] Tukey HSD was used as a post-hoc test with the data involving cartilage thickness, adiponectin, type X collagen, and PCNA. Tukey HSD was chosen because it assumes the equal variances of the groups, and it controls the type I error (false positive) well.[29] A difference of P < .05 was defined as statistically significant. All the measurements were performed blindly by one investigator. Cartilage thickness measurements were repeated after initial measuring, and intraclass correlation coefficient (ICC) was used to check the reliability of the measurements. ICC between initial measurements and repeated measurements was 0.972.

Results

Effect of Ovariectomy

Condylar cartilage thickness. The total thickness of condylar cartilage in the most superior and posterior segments was significantly larger in 67-day-old ovariectomized rats fed the soft food than in the control rats fed the soft food (P < .05) (Fig 2a). The thickness of condylar cartilage in the anterior, most superior, and posterior segments in the 87-day-old ovariectomized rats fed the normal food was significantly larger than in the control rats fed the normal food (P < .05) (Figs 2b and 3).

Number and size of osteoclasts. The 87-day-old ovariectomized rats in both food groups had a

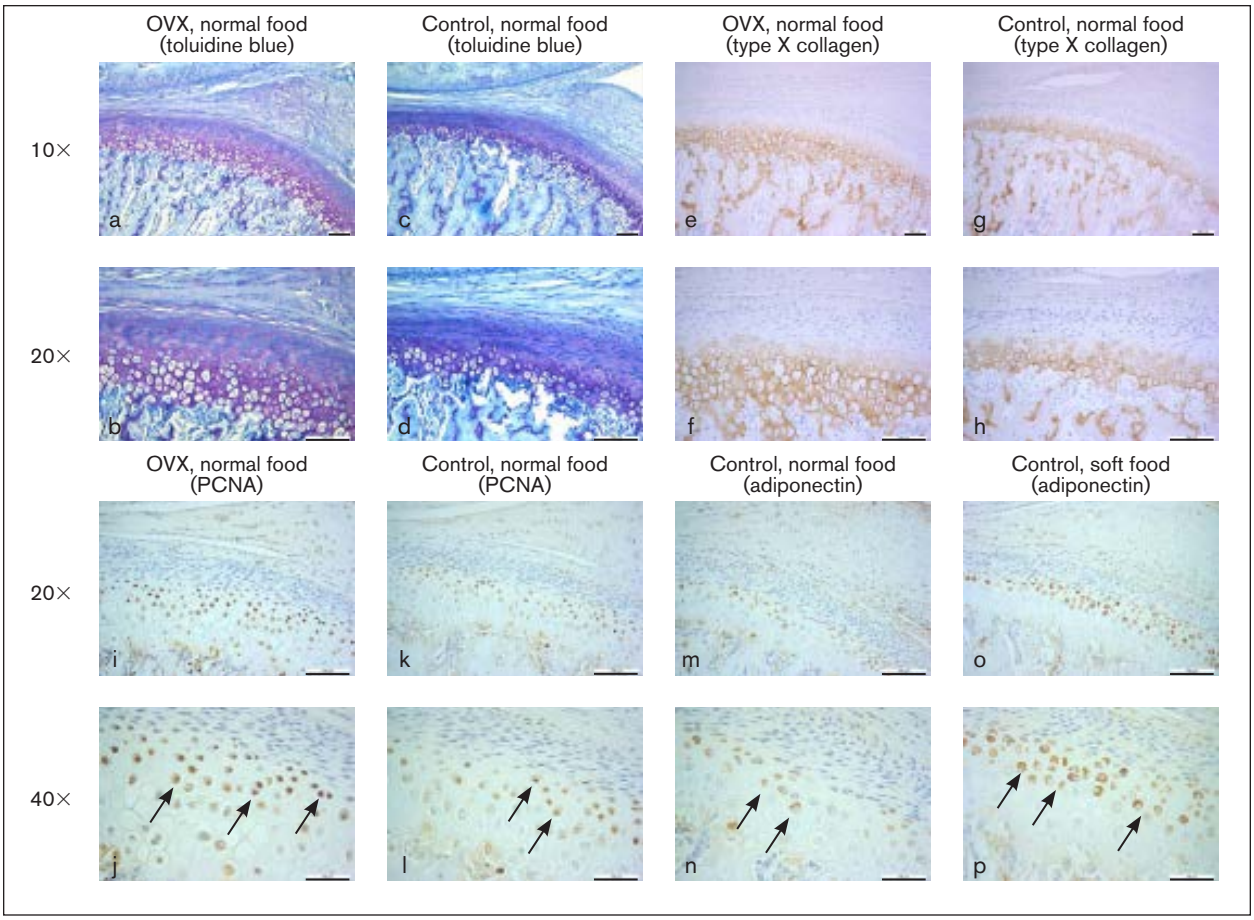


Fig 3 Histologic samples taken with three magnifications. **(a to h)** 10× upper row and 20× corresponding lower row. **(i to p)** 20× upper row and 40× corresponding lower row. All images present the most superior and posterior segment of condylar cartilage in 87-day-old rats. Toluidine blue staining of **(a and b)** an ovariectomized (OVX) rat fed the normal food and **(c and d)** a control rat fed the normal food; cartilage is clearly thicker in the ovariectomized rat than in the control rat. Immunostaining against type X collagen of **(e and f)** an ovariectomized rat fed the normal food and **(g and h)** a control rat fed the normal food; area stained positively against type X collagen is larger in ovariectomized rat when compared to control rat. Immunostaining against PCNA of **(i and j)** an ovariectomized rat fed the normal food and **(k and l)** a control rat fed the normal food. Immunostaining against adiponectin of **(m and n)** a control rat fed the normal food and **(o and p)** a control rat fed the soft food. Scale bar in 10× and 20× = 100 μm; scale bar in 40× = 50 μm. Arrows indicate the cells stained positively against antibody. The difference between specimen are clearly seen.

significantly lower number of osteoclasts than the corresponding control rats ($P < .05$) (Figs 4 and 5). The 67-day-old ovariectomized rats fed the soft food had significantly larger osteoclasts than the control rats ($P < .05$) (Fig 6). The average size of the osteoclasts in the 87-day-old ovariectomized rats was significantly smaller than in the control rats ($P < .01$) (Figs 5 and 6).

Expression of type X collagen. *Immunohistochemistry.* Type X collagen protein was localized in the extracellular matrix of chondroblastic and hypertrophic layers of condylar cartilage by immunohistochemistry. The 87-day-old ovariectomized rats fed the normal food had a significantly larger area expressing type X collagen than the control rats fed the normal food ($P < .001$) (Figs 3 and 7).

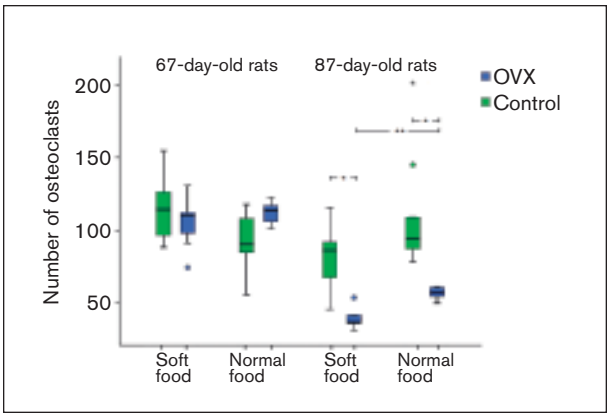


Fig 4 Box plot diagram illustrating the number of osteoclasts in 67- and 87-day-old rats. *$P < .05$; **$P < .01$. OVX = ovariectomized rats.

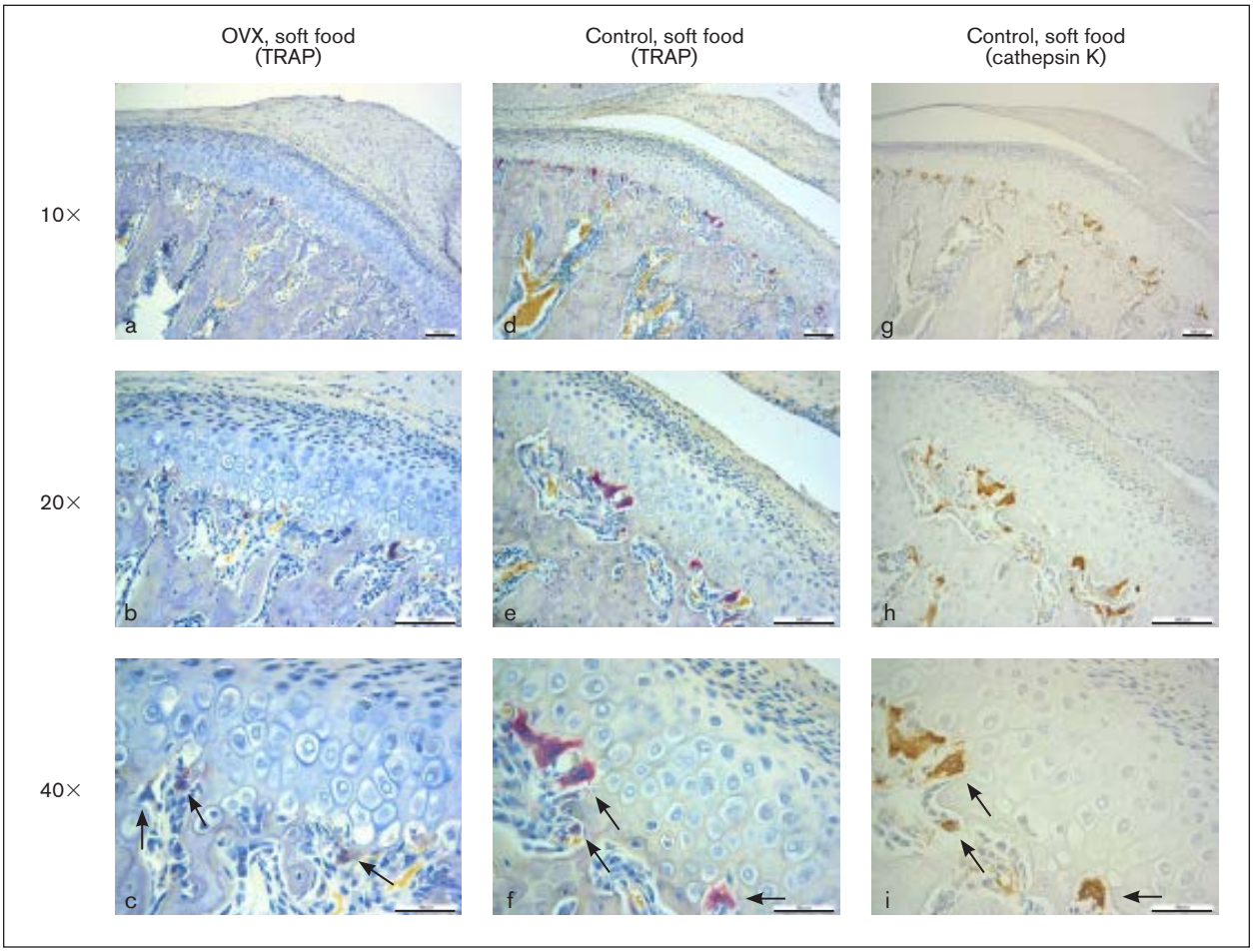


Fig 5 Histologic samples taken with three magnifications: 10× top row; 20× middle row; and 40× bottom row. All images present the most superior and posterior segment of condylar cartilage in 87-day-old rats. TRAP staining of **(a to c)** an ovariectomized (OVX) rat fed the soft food and **(d to f)** a control rat fed the soft food. **(g to i)** Immunostaining against cathepsin K of a control rat fed the soft food. Arrows indicate osteoclasts. The osteoclasts are smaller and fewer in number in a to c than in d to i. Both TRAP and cathepsin K staining were performed to ensure that the number and size of osteoclasts was registered accurately. Scale bar in 10× and 20× = 100 μm; scale bar in 40× = 50 μm.

In situ hybridization. Expression of type X collagen mRNA was localized by in situ hybridization to the hypertrophic chondrocytes. The type X collagen mRNA expression was higher in the ovariectomized rats fed the normal food than in control rats fed the normal food in both 67- and 87-day-old rats (Fig 8).

Proliferation and apoptosis. Both the 67- and 87-day-old ovariectomized rats fed the normal food had a higher proportion of PCNA-positive cells in the most superior and posterior segments of condylar cartilage than the control rats fed the normal food ($P < .001$, $P < .05$, respectively) (Figs 3 and 9). No difference in condylar cartilage apoptosis was found between the groups.

Effect of Altered Food Hardness

Condylar cartilage thickness. In the control group, the 87-day-old rats that were fed the normal food had a significantly thicker condylar cartilage than the rats that were fed the soft food ($P < .05$) (2b). The 87-day-old ovariectomized rats fed the normal food had a significantly thicker condylar cartilage in the anterior and most superior segments than the ovariectomized rats fed the soft food ($P < .05$) (Fig 2b).

Expression of type X collagen. The 67-day-old control rats fed the soft food had a significantly smaller area expressing type X collagen than the control rats fed the normal food ($P < .05$) (Fig 7). The 87-day-old control rats fed the soft food had a significantly larger area expressing type X collagen than the control rats fed the normal food ($P < .001$) (Fig 7). The 87-day-old ovariectomized rats fed a normal food had a significantly larger area expressing type X collagen than the ovariectomized rats fed the soft food ($P < .01$) (Fig 7).

Proliferation and apoptosis. The 67-day-old control rats fed the soft food had a higher proportion of PCNA-positive cells in the most superior segment

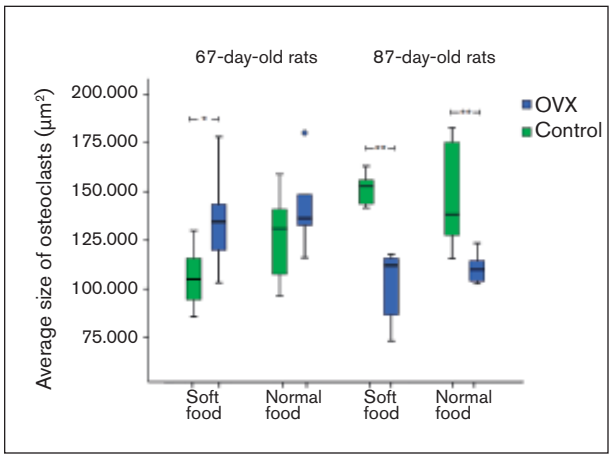


Fig 6 Box plot diagram illustrating the average size of osteoclasts in 67- and 87-day-old rats. *P < .05; **P < .01. OVX = ovariectomized rats.

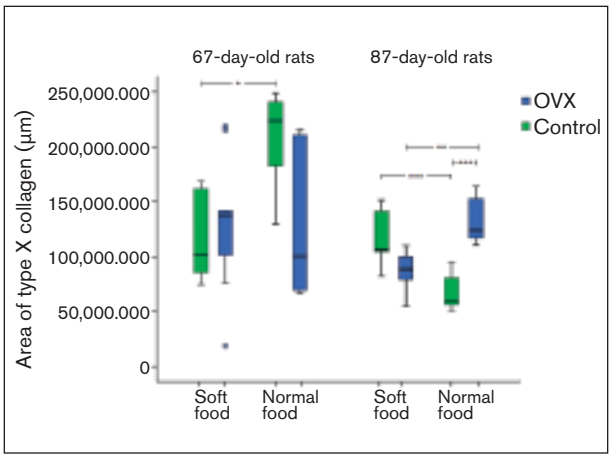
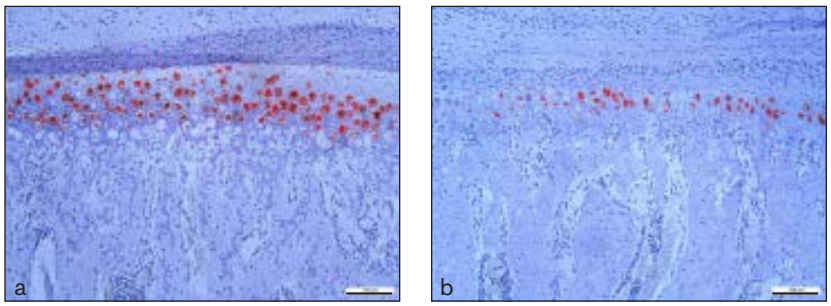


Fig 7 Box plot diagram illustrating the amount of type X collagen in condylar cartilage in 67- and 87-day-old rats. *P < .05; **P < .01; ***P < .001. OVX = ovariectomized rats.

Fig 8 In situ hybridization bright field image from the most superior segment of condylar cartilage with 20× magnification. A positive signal was obtained from the dark field image and then colored red. **(a)** An 87-day-old ovariectomized rat fed a normal food and **(b)** an 87-day-old control rat fed a normal food.



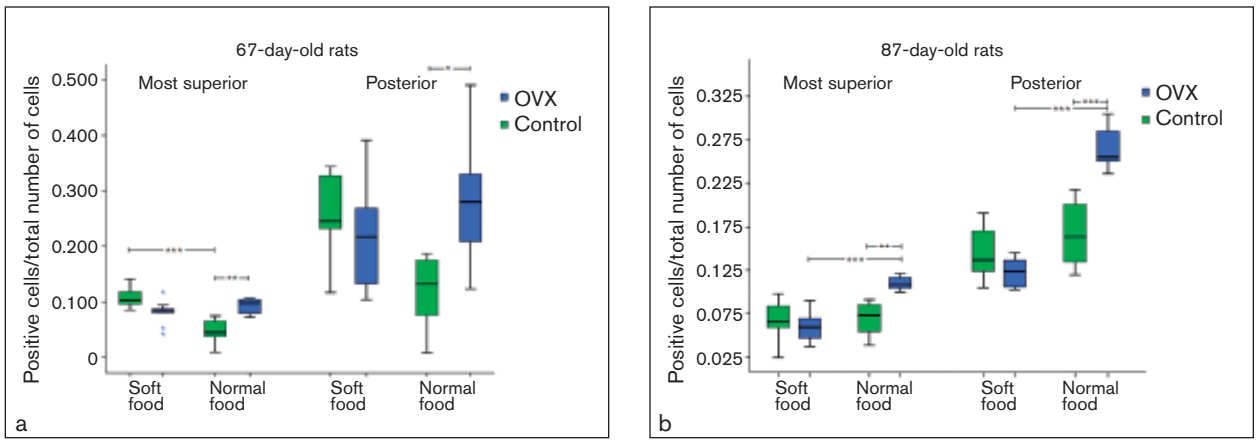


Fig 9 Box plot diagram illustrating the proportional amount of PCNA positive cells in condylar cartilage in **(a)** 67-day-old rats and **(b)** 87-day-old rats. *P < .05; **P < .01; ***P < .001. OVX = ovariectomized rats.

than the control rats fed the normal food (P < .001) (Fig 9a). The 87-day-old ovariectomized rats fed the normal food had a higher proportion of PCNA-positive cells in the most superior and posterior segments than the ovariectomized rats fed the soft food (P < .001) (Fig 9b). No difference in condylar cartilage apoptosis was found between the groups.

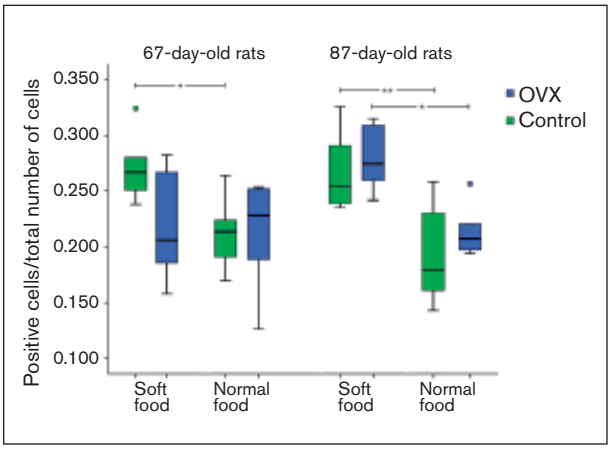


Fig 10 Box plot diagram illustrating the proportional amount of cells stained against adiponectin in condylar cartilage in 67- and 87-day-old rats. *$P < .05$; **$P < .01$. OVX = ovariectomized rats.

Adiponectin. In the posterior segment of the condylar cartilage, the 87-day-old ovariectomized rats fed the soft food had a higher proportion of positively stained cells than the ovariectomized rats fed a normal food ($P < .01$) (Fig 10). Both the 67- and 87-day-old control rats fed the soft food had a higher proportion of positively stained cells than the control rats fed the normal food ($P < .05$ in 67-day-old rats, $P < .01$ in 87-day-old rats) (Figs 3 and 10). No difference in adiponectin expression was found between the groups in the anterior and the most superior segments of the condylar cartilage.

Discussion

Condylar cartilage seems to be sensitive to both estrogen level and changes in food hardness. When estrogen is present, or soft food is administered, condylar cartilage remains relatively thin.

The condylar cartilage was significantly thinner in rats fed a soft food in both the control and ovariectomized rats. The effect of loading is at least partly transmitted to chondrocytes by transcription factors such as activator protein 1 (AP-1) and Runt-related transcription factor 2 (Runx2).[30] In addition, Sox-9 has been shown to enhance the differentiation of mesenchymal cells to chondrocytes and as a result increase the amount of cartilage matrix.[31]

Condylar cartilage is composed of fibrous and cartilage layers or zones beginning at the articular surface and ending at the underlying bone: articular, proliferative, chondroblastic, and hypertrophic cartilage zones.[32–34] The proliferative zone functions as a reserve for mesenchymal cells—chondrocyte precursors.[34] Chondrocyte precursors differentiate to mature chondrocytes, then grow in size to hypertrophic chondrocytes. The cartilage in the cartilage-bone interface is eventually replaced with endochondral bone and marrow.[35] Type X collagen is a marker for mature cartilage, and it is suggested that it acts as a replaceable material in the growth of bone matrix.[17] The present study showed very distinct expression profiles of type X collagen between younger and older rats. The older ovariectomized rats fed a normal food had a higher expression of type X collagen than the control rats. The explanation for this could be that condylar cartilage goes through maturation more quickly than it is replaced by bone. This theory is supported by the observed increase in cartilage thickness in the ovariectomized rats when compared with the control rats. Expression of type X collagen in the 67-day-old control rats fed a normal food was high compared with the other groups. This observation implies that type X collagen expression is temporarily down-regulated after an environmental change (ie, ovariectomy, food hardness), since the other three groups underwent an environmental change.

The number of osteoclasts was significantly lower and their average size was smaller in ovariectomized rats (ie, estrogen is not present) when compared with control rats. The observed increase in thickness of condylar cartilage in ovariectomized rats could have occurred because the osteoclasts were smaller and fewer in number—resorbing less calcified matrix and remnants of chondrocytes in cartilage-bone interface, which possibly could lead to a change in the balance of cartilage turnover. Despite the intensive research on estrogen and bone, the mechanisms by which estrogen regulates bone metabolism are still unclear.[7] It has been shown, however, that estrogen affects osteoclasts directly by suppressing receptor activator of nuclear factor kappa-B ligand (RANKL)-stimulated osteoclastic differentiation,[36] and indirectly by increasing the production of decoy receptor osteoprotegerin (OPG) for RANKL.[37]

It is unclear why the lack of estrogen did not increase the number of osteoclasts, as this could have been predicted from previous studies.[36] TRAP staining was applied to verify the positive staining of cathepsin K in osteoclasts, which reasserts the results of the present study. It has been shown that ovariectomy does not affect trabecular bone mass in the rat condyle but significantly decreases bone mass in secondary spongiosa in the rat femur.[38] The role of estrogen in bone metabolism is complex; it has been shown that when treating postmenopausal women with estrogen there is a temporary increase in bone formation markers followed by a sustained decrease.[7] It is noteworthy that the younger rats in the present study showed no significant differences in osteoclast number between experimental and control

groups, whereas significant differences were seen in the older rat groups.

Growth and maintenance of condylar cartilage depend on sufficient biomechanical stimuli. Previous studies have shown that reduced loading—for example, a soft food—affects cartilage metabolism and has a reducing impact on cartilage thickness. Loading of condylar cartilage by increased food hardness seems to be important for condylar cartilage growth and maintenance of ideal proliferation.[12,39] The present results have shown that proliferative activity, ie, PCNA expression, is higher in ovariectomized rats than in control rats, and ovariectomized rats fed the soft food had less proliferative activity than ovariectomized rats fed the normal food. It has been shown that additional estrogen inhibits proliferation of chondroblasts in rat mandibular condylar organ cultures.[9] The present study shows that ovariectomized rats fed a soft food had no significant differences in proliferative activity when compared with control rats fed a soft food. It seems that estrogen alone does not have a significant role in proliferative activity when food consistency is soft.

The staining pattern of PCNA showed great resemblance to the staining pattern of type X collagen in 87-day-old rats. Expression of type X collagen seemed parallel with the expression of PCNA. It is possible that the increased expression of type X collagen is a result of increased proliferation and turnover of cartilage, because the more cartilage cells there are, the more extracellular matrix and collagens are synthesized and decomposed. However, type X collagen might have an association with the response of condylar cartilage to proliferation inducers. Parathyroid hormone–related protein (PTHrP) promotes proliferation of chondroblasts and suppresses chondrocyte hypertrophy in epiphysial cartilage of long bone.[40–42] It has been shown that the area expressing type X collagen in cartilage of long bone in PTHrP-deficient mice is not affected, but in condylar cartilage the type X collagen area is reduced.[43]

Adiponectin is an adipocytokine synthesized and secreted into the bloodstream exclusively by adipose tissue, and it has many hormonal functions, such as taking part in glucose metabolism modulation.[44] Adiponectin acts via two receptors; one (AdipoR1) is expressed typically in skeletal muscle and is the only adiponectin receptor that is found in cartilage.[18,45] Adiponectin levels circulating in the bloodstream are decreased in obesity, and weight reduction can increase adiponectin levels.[44,46] In the present study, the proportional amount of cells stained against adiponectin was significantly higher in the soft-food groups than in the normal-food groups, in both the control and ovariectomized rats. Although high adiponectin levels are associated with good health and normal insulin resistance, there is evidence that adiponectin could act as a pro-inflammatory agent in skeletal joints.[18] It has been shown that adiponectin levels are abnormally high in rheumatoid arthritis.[47] The high proportional amount of cells stained positively against adiponectin and thinner condylar cartilage in rats fed the soft food could suggest that the change in condylar cartilage is a step toward a more pro-inflammatory phase or a similar event.

Chondrocytes play an important role in the synthesis and degradation of the articular matrix,[48] and therefore some effects of estrogen and loading might be transmitted to condylar cartilage via an increase or decrease in chondrocyte apoptosis. The results of the present study have shown that apoptosis probably plays no significant role in the changes in condylar cartilage caused by estrogen deficiency and altered food hardness. The number of apoptotic cells detected in condylar cartilage with the TUNEL assay and anti-p53 antibody was very low, and there was no difference in the number of apoptotic cells between groups. It has been shown that visible apoptosis is rare in relatively healthy condylar cartilage, whereas apoptosis is more prevalent, for example, in osteoarthrosis and experimentally created disordered occlusion.[49]

Conclusions

Condylar cartilage is thicker when estrogen is not present and food hardness is normal. It seems that estrogen deficiency leads to a decline in the number of osteoclasts and a diminishing of their size, which could partly explain the thicker condylar cartilage due to a slower resorption rate in the cartilage-bone interface. Adiponectin appears to be one important potential factor in transmitting the effect of loading, ie, food hardness on condylar cartilage. Ovariectomy seems to increase the proliferation of chondroblasts when food hardness is normal, and it increases the thickness of cartilage. The increased type X collagen expression in ovariectomized rats suggests that either estrogen deficiency speeds up the maturation of condylar cartilage, or the increased expression of type X collagen is a result of increased proliferative activity of cartilage cells. Both events could lead to thicker condylar cartilage, but further studies are needed to prove this. Further investigations are also needed to investigate the long-term effects of estrogen deficiency and altered food hardness on condylar cartilage.

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